

Maternal Thermal Effects on Female Reproduction and Hatchling Phenotype in the Chinese Skink (*Plestiodon chinensis*)

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Abstract We maintained gravid Chinese skinks (*Plestiodon chinensis*) at three constant temperatures (25, 28 and 31 °C) during gestation, and randomly assigned eggs from each female to one of the same three temperatures for incubation to determine maternal thermal effects on female reproduction and hatchling phenotype. Maternal temperature affected egg-laying date, hatching success and hatchling linear size (snout-vent length, SVL) but not clutch size, egg size, egg component, and embryonic stage at laying. More specifically, females at higher temperatures laid eggs earlier than did those at low temperatures, eggs laid at 31 °C were less likely to hatch than those laid at 25 °C or 28 °C, and hatchlings from eggs laid at 31 °C were smaller in SVL. Our finding that maternal temperature (pre-ovipositional thermal condition) rather than incubation temperature (post-ovipositional thermal condition) affected hatching success indicated that embryos at early stages were more vulnerable to temperature than those at late stages. Our data provide an inference that moderate maternal temperatures enhance reproductive fitness in *P. chinensis*.

Keywords Developmental plasticity, egg incubation, female reproduction, hatchling phenotype, maternal thermal effect, *Plestiodon chinensis*

1. Introduction

The thermal environments experienced by animals pervasively affect their physiological and behavioral performances (Villarreal *et al.*, 2007; Lepetz *et al.*, 2009; Goller *et al.*, 2015). Generally, thermal impacts are more pronounced in ectothermic species because their body temperatures are highly dependent on the environmental temperature. In reptiles, the thermal environments experienced by reproducing females affect not only oviposition or parturition date but also reproductive output and offspring phenotype (Sorci and Clobert, 1997; Shine and Downes, 1999; Ji *et al.*, 2006; Clarke and Zani, 2012; Wang *et al.*, 2014; Schwanz, 2016), although the

extent of these plastic responses to thermal environments varies among taxa, species, or populations. In lizards, maternal thermal effects on the phenotype of offspring are more noticeable in viviparous species than in oviparous species (Zhang *et al.*, 2010; Tang *et al.*, 2012; Wang *et al.*, 2014; Clarke and Zani, 2012; Lu *et al.*, 2013) and, within a species, such effects can be more significant in some populations but less so or even not in others (Du *et al.*, 2005; Luo *et al.*, 2010; Lu *et al.*, 2013; Ma *et al.*, 2014). To draw a general conclusion about this topic we need to collect data from more lizard species.

In this study, we maintained gravid female Chinese skinks [*Plestiodon* (formerly *Eumeces*) *chinensis*] at three constant temperatures [25, 28 and 31 (± 1) °C] to investigate maternal thermal effects on female reproductive traits (egg-laying date, post-ovipositional maternal body mass and condition, embryonic stage at laying, egg size, egg component, clutch size and clutch mass), egg hatching success and hatchling phenotype. This medium-sized (up to 134 mm snout-vent length,

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SVL), ground-dwelling scincid lizard is widely distributed in southeastern China (Lin and Ji, 2000; Lu *et al.*, 2014). Earlier studies of *P. chinensis* have showed the following: (1) female reproductive output can be affected by several factors (e.g., food availability, tail loss) and varies among populations and years (Ji *et al.*, 2002; Lu *et al.*, 2012, 2014); (2) embryonic development is highly sensitive to temperature, with embryonic mortality increasing dramatically at temperatures lower than 24 °C or higher than 32 °C (Ji and Zhang, 2001; Du *et al.*, 2005; Qu *et al.*, 2014; Shen *et al.*, 2017); and (3) females from Lishui, southwest Zhejiang province, where skinks used in this study were collected, lay a single clutch of 7–25 eggs per breeding season from early June to early July (Lu *et al.*, 2012, 2014). Although the temperature effect on embryonic development during the post-ovipositional period has been extensively evaluated in *P. chinensis*, little is known about the effect during the pre-ovipositional period. To fill this gap, we designed this study to evaluate maternal thermal effects on female reproductive traits and hatchling phenotype, and compare patterns observed in this species with those reported for other species so far studied.

2. Materials and Methods

2.1. Animal collection and husbandry We collected 120 adult *P. chinensis* (90 females and 30 males) larger than 85 mm snout-vent length (SVL) in late April 2004 in Lishui, Zhejiang, China (28°46'N, 119°92'E). Skinks were transported to our laboratory in Hangzhou, where 15 females and five males were randomly assigned to one of six terraria (length × width × height: 120 cm × 80 cm × 60 cm) filled with moist sand and grass.

We designed three temperature treatments, 25, 28 and 31 (± 1) °C, which were chosen because air temperatures between mid-April and June when female *P. chinensis* are gravid vary from 16 to 33 °C (Meteorological Bureau of Zhejiang Province) and temperatures optimal for *P. chinensis* embryos range from 26 °C to 30 °C (Ji and Zhang, 2001; Qu *et al.*, 2014; Shen *et al.*, 2017). Two terraria were placed in each of three AAPS (artificial atmospheric phenomena simulator) rooms at the corresponding temperatures [25, 28 and 31 (± 1) °C]. Two fluorescent lamps that suspended above each terrarium were switched on at 08:00 and off at 17:00. Mealworm larvae (*Tenebrio molitor*), house crickets (*Achetus domestica*) and water enriched with vitamins and minerals were provided *ad libitum*.

2.2. Female reproduction Females in the same

terrarium were isolated from each other using dividers (cuboids made by tin plate with two opposite sides open) that created 30 cm × 20 cm × 30 cm chambers soon before they laid eggs. We checked terraria at least twice daily for freshly laid eggs, thereby collecting and weighing eggs always less than 3 h post-laying. The viability of freshly laid eggs was judged by the presence of an embryonic disc using a spot light. Body mass, SVL and tail length were taken for each post-ovipositional female, and body condition was calculated as the residual from linear regression of ln-transformed body mass against ln-transformed SVL.

2.3. Embryonic stage and egg component at laying

One egg from each clutch was dissected to identify Dufaure and Hubert's (1961) embryonic stage at laying. Yolks from dissected eggs were placed in pre-weighed aluminum foil dishes. Eggshells were rinsed in water, and then dried by blotting with a paper towel. Yolks and eggshells were dried for 48 h in an oven at 65 °C, and weighed before and after drying.

2.4. Egg incubation and hatchling phenotypes A total of 422 eggs, 3–15 eggs from each clutch, were individually incubated in covered plastic jars (50 mL) filled with moist vermiculite at a water potential of –220 kPa (Wang *et al.*, 2013). Eggs from the same clutch were assigned evenly into three incubation temperature treatments [25, 28 and 31 (± 1) °C] to minimize family effects. Each egg was half-buried lengthwise in moist vermiculite. The egg surface near the embryo faced upwards and was exposed to air inside the jar. After weighing, incubation jars containing eggs were randomly assigned and placed on the shelves in the three fore-mentioned AAPS rooms. Each jar was weighed every other day, and distilled water was added into the substrate to compensate for loss due to evaporation and the absorption caused by the embryonic metabolism. Jars were moved among the shelves daily following a predetermined schedule to minimize any influence of thermal gradients inside the room.

We checked jars daily for newly hatched individuals, thereby collecting, weighing and measuring them a few hours post-hatching. We estimated the maximal sprint speed for each hatchling at a body temperature of 28 °C, which was achieved by placing a testing individual in an incubator (Sheldon MFG Inc, USA) at the correspondent temperature for a minimum of 30 minutes. Locomotor capacity was assessed by individually chasing down hatchlings along a 2 m racetrack with one side transparent, which allowed lateral filming with a Panasonic NV-GS408 digital video camera. Hatchlings

that refused to run were excluded from analyses. The tapes were later examined with a computer using MGI VideoWave III software (MGI Software Co., Canada) for sprint speed in the fastest 25 cm interval. All adults and hatchlings were released to the site of capture in late July.

2.5. Statistical analysis Prior to parametric analyses, data were tested for normality using the Kolmogorov-Smirnov test, and for homogeneity of variances using the Bartlett's test (at univariate level) or Box's M test (at multivariate level). One-way analysis of variance (ANOVA, for female SVL and post-ovipositional body condition) or analysis of covariance (ANCOVA with female SVL as the covariate, for clutch size, clutch mass and post-ovipositional body mass) was used to test for differences in female reproductive traits between maternal treatments. Mixed model ANOVA with maternal treatment as the fixed factor and clutch origin (female ID) as the random factor was used to test for differences in egg mass. Mixed model ANOVA or ANCOVA with maternal treatment and incubation temperature as fixed factors and clutch origin as random factor was used to test for differences in the length of incubation period, hatchling SVL, mass (with initial egg mass as the covariate), body condition, and maximal sprint speed (with hatchling SVL as the covariate), respectively. Multivariate analysis of variance (MANOVA) was used to test for differences in egg components (water content, yolk dry mass and eggshell mass) between maternal treatments. Kruskal-Wallis test was used to examine whether oviposition time and embryonic stage at oviposition differed between maternal treatments. Pearson Chi-squared (χ^2) tests were used to analyze the effect of temperature regime (or incubation temperature) on female reproductive success and egg-hatching success, respectively.

3. Results

3.1. Female reproduction Forty-five (50%) out of 90 females laid eggs between mid-May and early July. The proportion of females that laid eggs decreased as maternal temperature increased, with the differences marginally significant ($\chi^2 = 5.60$, $df = 2$, $P = 0.061$). Reproducing females assigned to different treatments did not differ from each other in mean SVL (Table 1). The duration of reproducing females that maintained under different thermal conditions before oviposition differed significantly, with fewer days for females under high temperature than those under low temperature (25 °C: 57.7 ± 1.8 days; 28 °C: 48.0 ± 2.3 days; 31 °C: 34.5 ± 1.8 days, $F_{2,42} = 31.17$, $P < 0.0001$). Females at 31 °C

laid eggs, on average, 13 days earlier than did those at 28 °C, and 23 days earlier than did those at 25 °C; the differences in egg-laying date were significant (Kruskal-Wallis ANOVA, $H_{2, N=45} = 26.01$, $P < 0.001$) (Figure 1). Egg mass varied among clutches ($F_{42, 636} = 34.90$, $P < 0.0001$) but not among treatments ($F_{2, 42} = 2.14$, $P = 0.130$). Neither clutch size nor clutch mass differed among treatments (Table 1). Maternal thermal effects on post-ovipositional maternal body mass and condition were significant, with mean values being both smaller at 25 °C than at 28 °C or 31 °C (Table 1).

3.2. Embryonic stage and egg component at laying Embryonic stages at laying ranged from Dufaure and Hubert's (1961) Stage 30 to 36, with mean values not

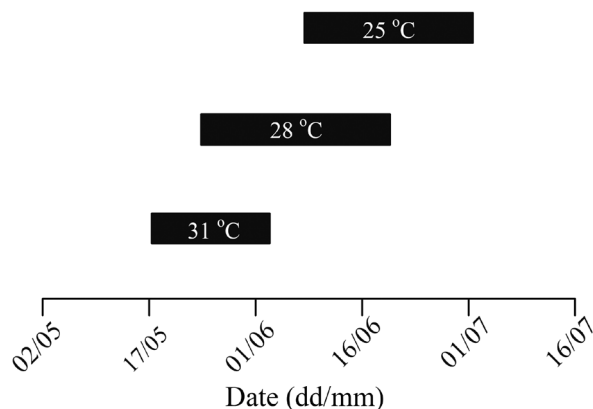


Figure 1 Egg-laying date of gravid female *Plestiodon chinensis* maintained at three constant temperatures.

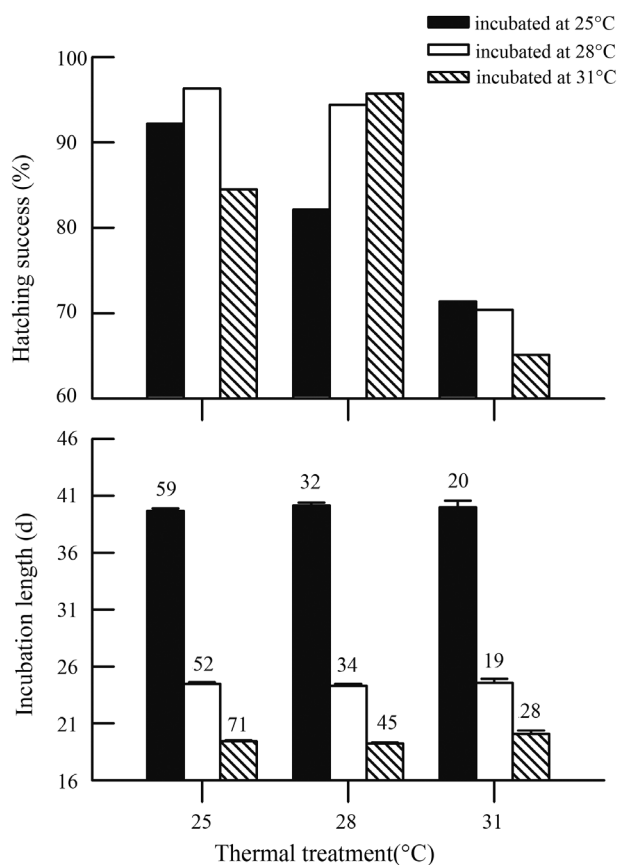
differing among treatments (Table 2). Eggs laid by females at different temperatures did not differ from each other in water content, yolk dry mass, or eggshell mass (Wilks' $\lambda = 0.88$, $df = 6$, 78 , $P = 0.516$; Table 2).

Initial egg mass did not differ among maternal treatments ($F_{2,42} = 2.27$, $P = 0.116$) and among incubation treatments ($F_{2,92} = 1.47$, $P = 0.235$). In this study, a total of 360 hatchlings were collected. Maternal temperature affected hatching success, with eggs laid at 31 °C being less likely to hatch than those laid at 25 °C or 28 °C ($\chi^2 = 29.28$, $df = 2$, $P < 0.001$). Incubation temperature did not affect hatching success ($\chi^2 = 2.77$, $df = 2$, $P = 0.250$) (Figure 2). Incubation length was affected by incubation temperature ($F_{2,99} = 4335.94$, $P < 0.001$) and clutch origin ($F_{42,75} = 4.22$, $P < 0.001$), but not by maternal temperature ($F_{2,41} = 0.40$, $P = 0.674$) or its interaction with incubation temperature ($F_{4,75} = 0.88$, $P = 0.480$) (Figure 2).

3.3. Hatchling traits Maternal temperature ($F_{2,38} = 7.11$, $P < 0.01$) and incubation temperature ($F_{2,58} =$

Table 1 Descriptive statistics, expressed as mean \pm SE and range, for reproductive traits of female *Plestiodon chinensis* maintained at three constant temperatures when gravid.

	Maternal temperature (°C)			Statistical results
	25	28	31	
<i>N</i>	20	14	11	
Maternal snout-vent length (mm)	100.7 \pm 1.2 91.8–108.9	101.9 \pm 1.5 91.2–111.1	99.2 \pm 1.7 89.5–107.0	$F_{2,42} = 0.68, P = 0.510$
Post-ovipositional maternal body mass (g)	18.6 \pm 0.6 15.3–22.1	20.5 \pm 0.9 14.3–26.3	19.8 \pm 0.6 16.4–22.3	$F_{2,41} = 6.52, P < 0.01$
Post-ovipositional maternal body condition	−0.044 \pm 0.016 −0.179–0.071	0.023 \pm 0.022 −0.113–0.119	0.050 \pm 0.022 −0.103–0.153	$F_{2,42} = 6.64, P < 0.01$
Clutch size	16.0 \pm 0.9 7–22	14.5 \pm 0.5 12–17	15.1 \pm 1.1 11–21	$F_{2,41} = 2.16, P = 0.129$
Clutch mean egg mass (g)	0.63 \pm 0.01 0.51–0.74	0.66 \pm 0.02 0.52–0.74	0.67 \pm 0.02 0.58–0.77	$F_{2,42} = 2.23, P = 0.121$
Clutch mass (g)	10.0 \pm 0.6 5.1–14.6	9.6 \pm 0.4 6.7–11.9	10.0 \pm 0.7 7.2–13.7	$F_{2,41} = 1.47, P = 0.241$

**Figure 2** Hatching success and incubation length of *Plestiodon chinensis* eggs laid and incubated at different temperatures. Numbers above the error bars are sample sizes.

3.73, $P = 0.030$) both affected hatchling linear body size (SVL), with hatchlings from eggs laid at 31 °C and incubated at 31 °C being smaller than those from eggs of other maternal \times incubation temperature combinations

(Figure 3). Neither maternal temperature nor incubation temperature affected hatchling mass or sprint speed (all $P > 0.111$) (Figure 3). The effects of clutch origin on hatchling SVL ($F_{41,42} = 1.68, P = 0.048$) and mass ($F_{42,67} = 2.69, P < 0.001$) were significant, but the effect on sprint speed was not ($F_{40,55} = 1.53, P = 0.073$). The effects of clutch origin \times incubation temperature interaction on the three examined hatchling traits were significant (SVL, $F_{51,68} = 1.74, P = 0.017$; mass, $F_{76,232} = 1.36, P = 0.042$; sprint speed, $F_{60,158} = 1.84, P < 0.01$).

We found that maternal temperature affected egg-laying date, post-ovipositional maternal body mass and condition, hatching success and hatchling phenotype in *P. chinensis*. Similar results have been reported for other oviparous lizards such as *Bassiana duperreyi* (Telemeco *et al.*, 2010), *Scincella modesta* (Lu *et al.*, 2013; Ma *et al.*, 2014), *Takydromus septentrionalis* (Luo *et al.*, 2010) and *Takydromus sexlineatus* (Lu *et al.*, 2018), although the impact on a given trait varies among species or populations. That warmer environments result in earlier oviposition is widespread in ectothermic species, simply because their body temperatures are highly dependent on the thermal environment to which they are exposed (Angilletta, 2009). High maternal temperatures in warm environments accelerate the pace of life (e.g., the rate of vitellogenesis and the rate of embryonic development as well), thus leading to earlier oviposition (Starostová *et al.*, 2012; Lu *et al.*, 2018). It is also possible that delayed oviposition results from extended egg retention (Braña and Ji, 2007; Telemeco *et al.*, 2010; Ma *et al.*, 2014).

Table 2 Descriptive statistics, expressed as mean \pm SE and range, for embryonic stage and egg composition in freshly laid eggs laid by female *Plestiodon chinensis* maintained at three constant temperatures when gravid.

	Maternal temperature (°C)			Statistical results
	25	28	31	
Embryonic stage	32.6 \pm 0.2 32–34	33.0 \pm 0.4 31–36	32.6 \pm 0.4 31–35	$H_{2, N=45} = 0.60, P = 0.739$
Egg mass (mg)	651.4 \pm 17.5 528.6–773.1	662.9 \pm 23.5 517.1–856.1	698.2 \pm 23.0 604.6–836.2	$F_{2, 42} = 1.20, P = 0.310$
Egg yolk dry mass (mg)	224.1 \pm 6.9 174.5–300.4	228.4 \pm 11.8 163.6–332.2	236.6 \pm 9.6 192.9–302.1	$F_{2, 42} = 0.44, P = 0.649$
Water content (%)	64.3 \pm 1.1 50.1–73.9	64.6 \pm 1.5 52.1–74.1	65.2 \pm 1.1 60.1–73.9	$F_{2, 42} = 0.08, P = 0.924$
Shell dry mass (mg)	7.9 \pm 0.2 6.2–9.6	7.6 \pm 0.3 6.2–9.6	7.6 \pm 0.1 6.7–8.2	$F_{2, 42} = 0.58, P = 0.564$

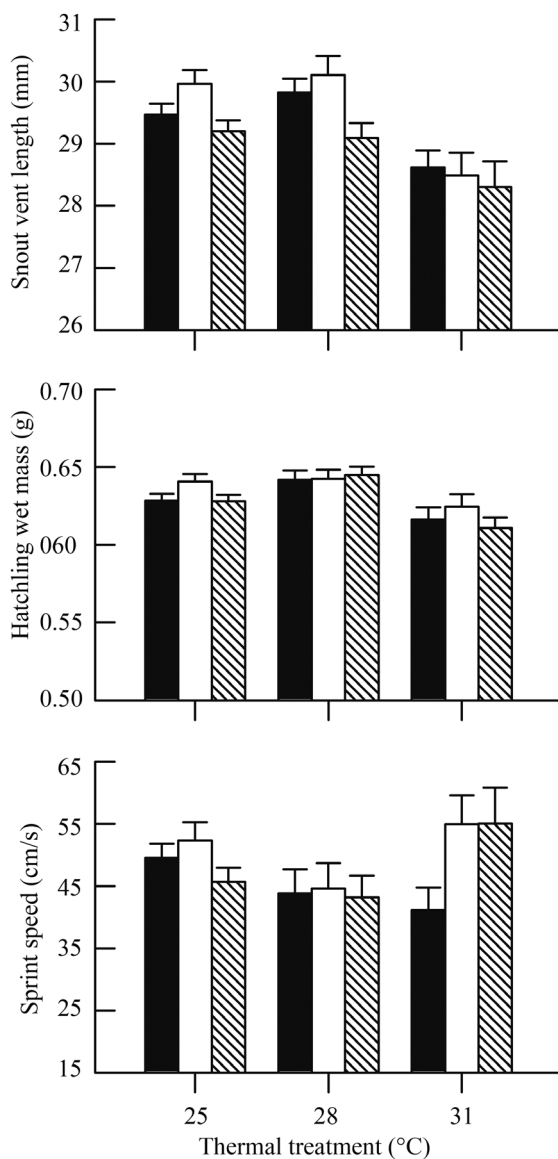


Figure 3 Body size (snout-vent length and body mass) and sprint speed of hatchling *Plestiodon chinensis* from eggs laid and incubated at different temperatures. See Figure 2 legend for explanation of barplots

However, this is not true for *P. chinensis*, as revealed by the fact that mean values for embryonic stage at laying did not differ among treatments. We also found that clutch origin and its interaction with incubation temperature had significant effects on hatchling traits, reflecting substantial contributions of maternal identity (probably including some genetic and non-genetic maternal components) to hatchling phenotypic variations (Warner *et al.*, 2008). Considering our aim of this study, we just focused on the effects of maternal thermal environments in the later discussion.

Population mean egg size of *P. chinensis* varies geographically but keeps remarkably constant within a population even females encounter substantial loss of stored energy (Lu *et al.*, 2012, 2014). These results, together with the finding that maternal temperature did not affect egg size, suggest that for a given population of *P. chinensis* eggs are well optimized for size and, unlike *S. modesta* (Ma *et al.*, 2014) and *T. septentrionalis* (Luo *et al.*, 2010) where females at high body temperatures lay smaller eggs in comparison with those at moderate body temperatures, egg size is not a thermally plastic life-history trait in the skink. One explanation for this discrepancy lies in that eggs laid by females at different temperatures differ in embryonic stage at laying in some species but not in others. For example, in contrast to that observed in *P. chinensis*, eggs laid by female *S. modesta* at 18 °C (low-temperature treatment) contain embryos at more advanced stages than those laid at 28 °C (high-temperature treatment) and are therefore greater in mass due to higher water content (Ma *et al.*, 2014).

It is common among reptiles that clutch size is more variable than egg size (Smith and Fretwell, 1974; Qu *et al.*, 2011). Earlier studies on oviparous lizards, including both single- and multiple-clutched species, consistently show that clutch size is a thermally less plastic life-

history trait (Du *et al.*, 2005; Luo *et al.*, 2010; Telemeco *et al.*, 2010; Clarke and Zani, 2012; Lu *et al.*, 2013, 2018; Ma *et al.*, 2014). Clutch size is determined soon before vitellogenesis (Jones and Swain, 2000). In field populations of *P. chinensis*, vitellogenesis begins no later than mid-April, and ovulation occurs no later than mid-May (Wang, 1966; Hu *et al.*, 2004). In this study, females were collected in late April when all decisions on clutch size had been made. It is therefore not surprising that mean values for clutch size did not differ among treatments.

Low hatching success observed in eggs produced by female *P. chinensis* at 31 °C suggests that exposure of embryos at early developmental stages to the temperature increases embryonic mortality. A similar result has been also reported for *S. modesta* and *T. sexlineatus* (Lu *et al.*, 2013, 2018). Earlier studies of *P. chinensis* showed that temperatures optimal for post-ovipositional embryonic development fall within the range of 26–30 °C, and exposure of eggs to temperatures higher than 30 °C may adversely affect embryonic development (Ji and Zhang, 2001; Qu *et al.*, 2014; Shen *et al.*, 2017). Here, our data provide further evidence that exposure of gravid females to temperatures higher than 30 °C also has adverse effect on embryonic development in the species. Embryonic lizards at early developmental stages are more vulnerable to high temperatures than those at late stages (Braña and Ji, 2007). This explains why maternal temperature rather than incubation temperature has a more important role in affecting hatching success in *P. chinensis*. It is predictable that the temperatures experienced by pre-ovipositional embryos should be lower than those by post-ovipositional embryos in the natural habitat of *P. chinensis*, because of lower air temperatures, and relatively lower and stabler preferred body temperatures selected by gravid females during gestation (Li *et al.*, 2012). Pre-ovipositional embryos might develop normally within a narrower temperature range compared with post-ovipositional embryos. Therefore, over an identical temperature range, maternal thermal effect on egg hatching success mattering more than incubation effect was not so surprising.

Our finding that embryonic stages at laying did not differ among treatments is consistent with the results reported previously for the same population (Lu *et al.*, 2012, 2014). In lizards, egg retention may be prolonged beyond the time when oviposition normally takes place often in dry or cold environments not suitable for nesting (Telemeco *et al.*, 2010; Ma *et al.*, 2014). In *S. modesta*, for example, females at 18 °C (low-temperature treatment) lay eggs with more developed embryos (Ma

et al., 2014) but, at 20 °C, they lay eggs with embryos at embryonic stages similar to those laid by warm-treated females (Lu *et al.*, 2013). Between mid-April and June, the daily mean air temperature in Lishui varies from 16 to 33 °C, with a mean value of 25.5 (\pm 0.4) °C. Most of the time, the temperature of 25 °C is not lower than the mean temperature experienced by most gravid females in nature, thus not being low enough to cause females to prolong egg retention. This explains why mean values for embryonic stage and incubation length did not differ among treatments. Eggs laid by females at low temperatures take a shorter time to hatch in species where low temperatures increase embryonic stages at laying (Lorion *et al.*, 2012; Ma *et al.*, 2014), or a longer time to hatch in species where low temperatures retard embryonic development (Du *et al.*, 2005; Telemeco *et al.*, 2010).

In this study, maternal temperature and incubation temperature appeared to have a similar impact on hatchling SVL, with linearly smaller hatchlings derived from eggs laid at 31 °C and incubated at the same temperature. Hatchlings from eggs incubated at high temperatures often have a greater amount of unused yolk and are therefore smaller in SVL than those from eggs incubated at relatively low or moderate temperatures (Ji *et al.*, 1999; Ji and Zhang, 2001). Neither maternal temperature nor incubation temperature affected hatchling body mass, presumably because total energy and material (such as yolk proteins, maternal hormones) allocated by females into individual eggs or transferred from egg to hatchling was similar between treatments. Such discrepancy of thermal effects on hatchling SVL and body mass has been reported in other studies on lizards (Ji and Braña, 1999; Clarke and Zani, 2012; Ma *et al.*, 2014). For example, in *S. modesta*, heavier but not longer hatchlings emerged from larger eggs laid by cold-treated females than those by warm-treated females (Ma *et al.*, 2014).

Despite limited impact on hatchling size and locomotor performance, maternal temperature substantially affects egg-laying date and hatching success. Hatching earlier at high temperatures prolong the time of growth before the onset of hibernation (Olsson and Shine, 1997; Warner and Shine, 2007). However, low hatching success highlights the adverse effect of high temperature on embryonic development (Shine and Harlow, 1993; Lu *et al.*, 2013). Data of this study, together with those reported in other studies addressing maternal thermal effects on offspring phenotype and fitness (Lourdais *et al.*, 2004; Chamaille-Jammes *et al.*, 2006; Lu *et al.*, 2013, 2018; Ljungström *et al.*, 2015), allow us to conclude that moderate maternal temperatures enhance reproductive fitness in lizards.

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